

## SIZE AND DISTRIBUTION OF RESTING EGGS IN A NATURAL POPULATION OF THE ROTIFER *BRACHIONUS PLICATILIS*

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**ABSTRACT** Resting eggs of the rotifer *Brachionus plicatilis* were collected from the sediments of a brackish-water pond near Tampa, Florida. These core samples were examined for resting egg density and size. Resting egg density decreased exponentially from the surface down to 7 cm in the sediments at all stations, with depth in the sediments accounting for 42.3% of the total variance. The maximum resting egg density recorded was 194 RE/cm<sup>3</sup> with no significant differences in density among stations. Resting eggs were found to be significantly larger in the top 2 cm of sediment than resting eggs from deeper in the sediments. These data are discussed with regard to other work on resting egg densities in natural populations. Hypotheses are offered to explain the observed decrease in resting egg size with depth in sediments.

### INTRODUCTION

The life cycle of monogonont rotifers is one of cyclical parthenogenesis (Birky and Gilbert 1971), with sexual reproduction occurring intermittently in response to specific environmental cues (Gilbert 1977, Pourriot and Rougier 1979, Pourriot and Clement 1981). The products of sexual reproduction are resting eggs which have a resistant outer shell and are capable of remaining dormant for up to several years (Gilbert 1974, Pourriot and Snell 1983). Resting eggs are energetically costly to make and, as a result, females producing resting eggs exhibit low net fecundity (Gilbert 1980). However, because of their capacity for extended dormancy, it is theoretically possible for resting eggs to accumulate in the sediments over several years reaching quite high densities.

The few reports on resting egg densities in natural populations (Ito 1958, Nipkow 1961) have suggested that resting eggs may indeed be present in the sediments of lakes and ponds in substantial quantities. As a result, we decided to investigate the density and distribution of resting eggs of the brackish-water rotifer *Brachionus plicatilis* in a pond known to support large and persistent adult populations. Secondly, we have examined resting egg size and its variation with resting egg distribution.

### MATERIALS AND METHODS

Resting eggs of the brackish-water rotifer *Brachionus plicatilis* were collected from the bottom sediments of a small, brackish-water pond (Westshore Pond), in Tampa, Florida. This approximately 100-m-diameter pond is connected to Tampa Bay by a narrow canal about 1 km long. The pond exhibited small tidal fluctuations and ranged from 10 ppt to 28 ppt in salinity and from 10°C to 33°C in temperature throughout the year. The sediments consisted primarily of mud rich in organic material and detritus. Because Westshore is a small pond, the sediment was rather homogeneous throughout, without any noticeable differences

between stations.

Ten-cm-diameter bottom cores were taken from five stations at various sites around the pond. From each core sample, three replicate subcores, 1.5-cm-diameter by 7-cm-deep, were removed for analysis. Each subcore was then sliced into 1-cm sections, according to depth, and the resulting 1.5-cm by 1-cm sections were filtered through a sieve series of 1024- $\mu$ , 512- $\mu$ , 256- $\mu$ , 128- $\mu$ , and 64- $\mu$  mesh. To determine resting egg density, the residue on the 64- $\mu$  sieve was washed into a small beaker with 25 ml of 1.75-M sucrose solution. This was followed by a second wash with 5 additional ml sucrose. The mixture of residue and sucrose solution was then poured into 1-ml counting chambers and the resting eggs were counted at 25X magnification under a stereomicroscope.

One of the major difficulties in counting resting eggs is separating them from debris in the sediments. Because they are small (110 x 70 $\mu$ ), resting eggs are easily obscured by sand grains and other particles. The use of a high molarity sucrose solution causes resting eggs to float and facilitates more accurate counting. Utilizing the sugar flotation technique, we were able to recover substantially more resting eggs from the sediments than by sieve series filtration alone.

Resting eggs used for size measurements were not collected by sugar flotation. All eggs used for size determination were only filtered through a sieve series and then washed from the 64  $\mu$  mesh filter with a 22 ppt salinity solution, the same salinity prevailing at the time of their collection from Westshore Pond. At least ten eggs were collected from each of the seven 1.5 x 1-cm sections, which were categorized according to depth. This was repeated for each of the three replicate subcores at Station C. In addition, eggs were also collected by filtration from the top 2 cm of the three replicate subcores of the other four stations. This permitted us to investigate resting egg size variability with depth at Station C as well as spatial variability in size among all stations in the top 2 cm of sediment. The length and width of each egg was measured with an ocular micrometer at 50X magnification with a Wild M5A stereomicroscope.

## RESULTS

The highest resting egg densities were observed in the top 1 cm of sediment (Figure 1), with the maximum being  $356 \pm 50.4$ /section ( $194/\text{cm}^3$ ) at Station E. From the top 1 cm, resting egg density decreased exponentially with depth, until finally, at some stations no resting eggs could be detected in the 7 cm deep section.

Spatial variation in resting egg density was also examined among stations. The mean density at a station ranged from  $40.8$  eggs/ $\text{cm}^3$ , including surface to 7 cm depth, to  $10.4$  eggs/ $\text{cm}^3$ . A two-way ANOVA (Sokal and Rohlf 1969, p. 315) was performed on these data to partition the variation into depth, station, and interaction effects. Depth as well as the depth x station interaction make significant contributions to the variance ( $P < .001$ ), however, station contributions are non-significant. An examination of the variance components (Sokal and Rohlf 1969, p. 317) reveals that depth accounts for 42.3%, station 4.7%, depth x station interaction 44.1%, and error 8.9% of the total variance.

Resting egg size, in both length and width dimensions, was found to vary with depth in sediments (Figure 2). Resting eggs collected from the top 2 cm were approximately 10% longer and 14% wider than eggs collected deeper. The results of Duncan's Multiple Range Test (Steel and Torrie 1960, p. 107) show that only eggs collected from the top 2 cm are significantly longer than eggs collected deeper (Table 1). A similar pattern exists with egg width.

A one-way analysis of variance (Sokal and Rohlf 1969, p. 208) of resting egg size in the top 2 cm of sediment among the five stations reveals small, but significant spatial heterogeneity. The F-values obtained were 3.191 ( $P < .05$ ) and 9.16 ( $P < .005$ ) for length and width respectively. Among the five stations, mean resting egg length varied about 10% from 99 to  $110\mu$  and mean width varied about 18% from 61 to  $75\mu$ .

## DISCUSSION

The resting egg densities we observed were similar to those reported by Ito (1958) and Nipkow (1961). Examining the sediments of 15 eel-culture ponds in Japan, Ito (1958) collected sediment samples to a depth of 5.5 cm. He reported densities of *Brachionus plicatilis* resting eggs as high as  $9.66/\text{mm}^2$ , with large variations between ponds. In contrast to our results, Ito found no consistent differences in resting egg density with depth in sediment. It should be recalled, however, that we used the sugar flotation technique to determine resting egg density, while Ito apparently simply picked resting eggs from the sediments. Ito also reported the interesting observation that resting egg densities in sediment surface samples were positively correlated with the population density of *B. plicatilis* in the summer of the preceding year.

Resting egg densities in sediments of eight planktonic rotifer species have been reported by Nipkow (1961). He

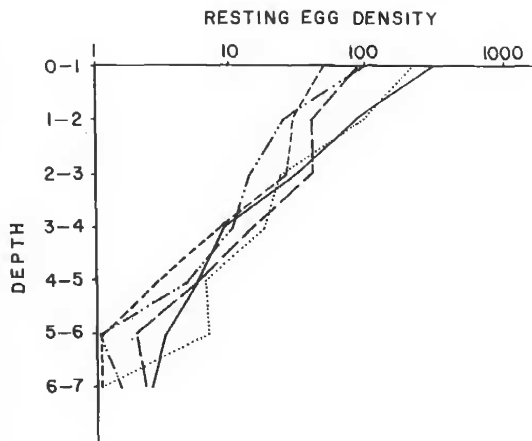


Figure 1. Resting egg density in sediments. Density is in resting eggs/section (1.5 cm diameter x 1 cm deep), depth is in cm. Each of the five stations is represented by a different line.

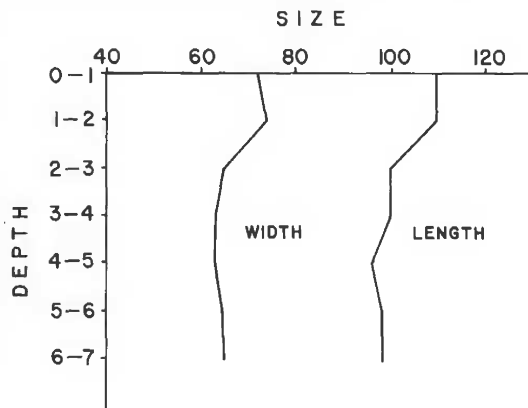


Figure 2. Resting egg size at various depths in sediments. Size is in microns, depth is in cm.

found surface sediment densities ranging from 1 to 6 resting eggs/ $\text{mm}^2$  for seven of the species, but densities between 30 and  $40/\text{mm}^2$  for *Synchaeta oblonga*. When we convert our data on *B. plicatilis* resting egg densities in the surface sediments to similar units, we obtain maximum estimates of two resting eggs/ $\text{mm}^2$ . These results are similar to Nipkow's in spite of the fact that we used different collecting techniques.

Our observations, along with those cited above, clearly suggest that a rotifer resting egg pool in the sediments can be of substantial size. The existence of this pool acts as a buffer for rotifer populations during periods of environmental unsuitability. The resting egg pool provides a vehicle for rapid recolonization when conditions become favorable again for growth.

TABLE 1.  
Results of Duncan's Multiple Range Test on resting egg length and width. Table entries are the mean lengths and widths in microns at each depth.

Length						
110	110	100	100	99	99	96
Width						
74	71	65	64	64	63	63

Species specificity in resting egg size has been recorded by several authors. Nipkow (1958) observed eggs collected from natural populations of *Brachionus calyciflorus* and *B. angularis* to be 144–160 x 88–104  $\mu$  and 80–96 x 60–64  $\mu$ , respectively. The most extensive comparison of resting egg dimensions has been provided by Nipkow (1961) who lists resting egg dimensions for 45 rotifer species. The range includes the smallest resting eggs, those produced by *Cephalodella exigua* at 38 x 40  $\mu$  to the largest—*Brachionus calyciflorus* at 144–160 x 88–104  $\mu$ . Similar dimensions for *B. calyciflorus* resting eggs were reported by Bogoslavsky (1963). Pourriot (1967) has reported considerably larger resting eggs in two species of rotifers cultured in the laboratory. He found very large resting eggs in *Notomata collaris*

(215 x 170  $\mu$ ) as well as *Tetrasiphon hydrocora* (200 x 155  $\mu$ ). Females in both these species are large, ranging up to 650  $\mu$ . Pourriot also reported the resting eggs of *Lindia torulosa* (120 x 90  $\mu$ ) and *Platyias polyacanthus* (132 x 118  $\mu$ ) to be somewhat smaller, but within the range reported by Nipkow (1961).

The *B. plicatilis* resting eggs that we collected from West-shore Pond were intermediate in size at 96–110 x 63–74  $\mu$ . We also found significantly smaller eggs 2 cm or more into the sediments. Why would resting eggs from deeper in the sediments be smaller? There are at least three hypotheses that could explain this observation. The first is that greater osmotic pressures may exist deeper in the sediments, causing shrinkage of the extraembryonic space usually present in resting eggs (Wurdak et al. 1978). A second alternative is that deeper eggs could be older and, as a result of their age, be smaller from partial metabolism of energy stores within the egg. A third alternative is that size-selective sorting of resting eggs could have occurred causing the larger, more buoyant eggs to be retained toward the sediment surface and smaller, denser eggs to penetrate deeper into the sediments. Distinguishing among these hypotheses will require further investigation of resting egg structure, physiology and interaction with the sediments.

#### ACKNOWLEDGMENTS

This research was supported in part by Grant No. 8003604 from the National Science Foundation and by the United States-Israel Binational Agricultural Research and Development Fund (BARD).

#### REFERENCES CITED

- Birky, C. W. & J. J. Gilbert. 1971. Parthenogenesis in rotifers: The control of sexual and asexual reproduction. *Am. Zool.* 11:245–266.
- Bogoslavsky, A. S. 1963. Materials to the study of the resting eggs of rotifers. Communication I. (Rus). *Byull. Mosk. Ova. Ispyt. Prir. Otd. Geol.* 68:50–67.
- Gilbert, J. J. 1974. Dormancy in rotifers. *Trans. Am. Microsc. Soc.* 93:490–513.
- . 1977. Mictic-female production in monogonont rotifers. *Arch. Hydrobiol. Beih.* 8:142–155.
- . 1980. Female polymorphism and sexual reproduction in the rotifer *Asplanchna*: Evolution of their relationship and control by dietary tocopherol. *Am. Nat.* 116:409–431.
- Ito, T. 1958. Studies on the "Mizukawari" in eel-culture ponds. X. The density of dormant eggs of rotifer on bottom deposits in eel-culture ponds. *Rep. Fac. Fish. Prefect. Univ. Mie.* 3:170–177.
- Nipkow, F. 1958. Beobachtungen bei der Entwicklung des Dauereies von *Brachionus calyciflorus* Pallas. *Schweiz. Z. Hydrol.* 20:186–194.
- . 1961. Die Radertiere im Plankton des Zurichsee und ihre Entwicklungsphasen. *Schweiz. Z. Hydrol.* 23:398–461.
- Pourriot, R. 1967. Males et oeufs durables de quelques Rotiferes. *Bull. Soc. Zool. Fr.* 92:185–192.
- & C. Rougier. 1979. Influences conjuguees du groupement et de la qualite de la nourriture sur la reproduction de *Brachionus plicatilis* O. F. Muller (Rotifere). *Neth. J. Zool.* 29(2):242–264.
- Pourriot, R. & P. Clement. 1981. Action de facteurs externes sur la reproduction et le cycle reproducteur des Rotiferes. *Acta. Oecol., Oecol. Gen.* 2:135–151.
- Pourriot, R. & T. W. Snell. 1983. Resting eggs in rotifers. *Hydrobiologia*.
- Sokal, R. R. & F. J. Rohlf. 1969. *Biometry*. W. H. Freeman and Company, San Francisco.
- Steel, R. G. D. & J. H. Torrie. 1960. *Principles and Procedures of Statistics*. McGraw-Hill, New York.
- Wurdak, E. S., J. J. Gilbert & R. Jagels. 1978. Fine structure of the resting eggs of the rotifers *Brachionus calyciflorus* and *Asplanchna sieboldi*. *Trans. Am. Microsc. Soc.* 97:49–72.